REMARKS/ARGUMENTS

Claims 79 - 99 were previously pending. Claims 85 to 90 are herein canceled without prejudice. Claims 79 to 81 are amended. After entry of these amendments, claims 79 to 84 and 91 to 99 will be pending.

Amendments to the claims

79 to 81 were amended to set forth that the donor, linker, and acceptor moiety are fused in a single amino acid sequence. Support for this subject matter is found *inter alia* in the specification at page 12, lines 23 to 28 and in the previous version of the claims. The base claims were also amended to set forth that the linking moiety consists of between 5 to 50 amino acids. Support for this recital is found in canceled claims 85 to 87.

Accordingly, the Applicants believe the amendments to the claims add no new matter and respectfully request their entry.

Response to the rejection of claims 79 to 81 and 94 to 96, and 98 for an alleged lack of written description under 35 U.S.C. §112.

The base claims each set forth in part a tandem *Aequorea*-related fluorescent protein in which both fluorescent moieties contain, as defined in the detailed description of the invention, "...any contiguous sequence of 150 amino acids of the fluorescent protein (that) has at least 85% sequence identity with an amino acid sequence, either contiguous or non-contiguous, from the wild type *Aequorea* green fluorescent protein..." (see the paragraph bridging pages 14 and 15 of the application). An *Aequorea*-related fluorescent protein moiety (herein "*Av*GFP-rp") would therefore encompass any polypeptide having the recited substitutions, with fewer than than 23 amino acid differences in any contiguous 150 residues with respect to the wild type protein of SEQ ID NO:2.

The Action posits two contributing reasons for sustaining the rejection for an alleged lack of written description of the 'at least 85% sequence identity' subject matter. The first reason concerns the possibility that, given such breadth, some of the envisioned constructs might

lack the requisite fluorescent activity. The second reason concerns the absence of a representative number of species within the gambit of the 85% sequence identity recitals. Applicants address both these concerns.

As noted by the Examiner, the Federal Circuit court of Appeals addressed the description adequate to show one of skill that the inventors were in possession of a claimed genus at the time of filing. *See, e.g., Enzo Biochem, Inc. v. Gen-Probe, Inc.*, 63 USPQ2d 1609 (Fed. Cir. 2002). An applicant may also show that an invention is complete by

> . . . disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that applicant was in possession of the claimed invention . . . *i.e.*, complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics. *Id.* at 1613.

Furthermore, "description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces." *See, e.g.*, 66 Fed. Reg. 1099, 1106 (2001). "In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus." *University of California v. Eli Lilly & Co.*, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). M.P.E.P. 2163.

Applicants standby their earlier remarks without further repeating them here. The Applicants provide herein a more comprehensive analysis to show that the combination of cited art and knowledge in the field, prior to the priority filing date, together with the instant specification would lead one of ordinary skill in the art to understand that the Applicants were in possession of constructs of an *Av*GFP-rp having up to 54 mutations, including constructs having up to 40 mutations in a contiguous stretch of 150 amino acids between wild type residues 89 and 238, that would retain some useful, if not necessarily improved, fluorescent activity.

The specification clearly discloses the relevant identifying characteristics, structure or other physical and/or chemical properties, by functional characteristics coupled with known or disclosed correlation between function and structure, or by combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. The specification incorporated by reference all the publications cited therein by virtue of the operation of the paragraph bridging pages 45 and 46. At p. 14, line 24, the specification sets forth and incorporates by reference a journal article which dissects the maturation of the GFP fluorophore at the molecular level (*see,* Heim et al. *PNAS-USA* 91:1201 (1994) (already of record)). With respect to structure, the specification incorporates by reference at line 27 on page 14 the disclosure of U.S. Patent Application Serial No. 08/706,408 which matured as U.S. Patent No. 6,124,128 (enclosed with IDS). The '408 application discloses in Figures 1 to 5AT the high-resolution crystal structure of an AvGFP-rp, in this case the wild type protein. The specification further discusses how to use the disclosed structure to identify amino acids whose mutation would alter the fluorescent properties of the protein. By corollary, one of ordinary skill in the art would appreciate that the specification further discloses how to use the disclosed structure to identify the many more amino acids whose conservative substitutions would **not** be likely to alter fluorescent properties of the protein.

The specification itself provides a table containing a number of mutational combinations that were engineered for specific utilities, be they shifted excitation and or emission spectra, increased quantum yields, greater relative fluorescence, or otherwise (see Table I on page 16). Clone W2, found in said table, consists of an AvGFP-rp with seven specific mutations; Y66W, I123V, Y145H, H148R, M153T, V163A, and N212K, of which six fall between residues 89 and 238. Similarly, the '505 specification incorporates by reference at line 25 on page 14 the disclosure of U.S. Patent Application Serial No. 08/337,915 which sets forth seven mutations; K3R, D76G, F99I, N105S, E115V, T225S, and K238E which had *no* significant impact on the properties of their corresponding Aequorea green fluorescent proteins (see, U.S. Patent No. 5,625,048, already of record and which matured from U.S. Patent Application Serial No. 08/337,915, at col. 5, second full paragraph, lines 47 to 54). The '048

patent also discloses that minor deletions at either end of the protein can be made with little or no impact on the fluorescent spectrum of the protein.

Furthermore, in the first full paragraph on page 20, the specification identifies other sites in which complementary mutations can be generated to promote binding interactions between two GFPs. For instance, it teaches that amino acids could be changed to positively charged amino acids in one fluorescent protein (for example Lys or Arg) and to negatively charged amino acids in the second fluorescent protein of the construct (for example Glu or Asp) thereby creating additional electrostatic interactions between two GFPs. In particular, the specification sets forth on page 20 that amino acids Y39, E142, N144, N146, S147, N149, Y151, R168, N170, E172, Y200, S202, Q204 and S208 are suitable locations for making such changes. Importantly, 13 of these 14 residues fall between residues 89 and 238. Persons of ordinary skill would also recognize that such locations would not be critical to the operability of the protein as a fluorescent protein and would be locations where conservative substitutions would be especially well tolerated with respect to retaining fluorescent activity.

Additionally, the incorporated disclosure of the '128 patent discloses eight additional residues; T44A, F64L, V68L, S72A, N146I, I167T, S175G, and S205T, referred to as "folding mutations", which "improve the ability of fluorescent proteins to fold at higher temperatures, and to be more fluorescent when exposed in mammalian cells, but have little or no effect on the peak wavelengths of excitation and emission."(see, U.S. Patent No. 6,124,128 at column 12, lines 55 to 60). In this passage, the '128 patent discloses that these mutations are contemplated for use with other mutations affecting the chromophore. Additionally, the '505 specification teaches protein variants with reduced hydrophobicity at A206, L221, or F223 (see, p. 20, lines 6 and 7). For example, replacement of Ala 206, or Leu 221 by any of the amino acids, Val, Ile or Phe would increase their hydrophobicity, and potentially strengthen the hydrophobic interaction between two GFPs.

Taken together, the specification therefore provides support for a variant of an AvGFP-rp, consisting of up to 38 mutations (residues 3, 39, 44, 64, 66, 68, 72, 76, 99, 105, 115, 123, 142, 144, 145,146, 147, 148, 149, 151, 153, 163, 167, 168, 170, 172, 175, 200, 202, 204,

205, 206, 208, 212, 221, 223, 225, and 238), with respect to the wild type sequence (84% sequence identity), of which 30 fall between the 150 contiguous residues stretching from 89 and 238 (80% sequence identity), and 31 of which fall between the 150 contiguous residues stretching from 63 to 212 (79% sequence identity). Importantly, , the mutations added to this AvGFP-rp, with respect to the clone W2 construct, are taught (as discussed above) as having little or no effect on the peak wavelengths of excitation and emission and provide polypeptides which have properties essentially or substantially indistinguishable from those of the specific polypeptide disclosed herein.

Furthermore, additional art which was incorporated by reference at page 14, line 23 of the '505 specification discloses several other silent mutations with respect to the peak wavelengths of excitation and emission. Prasher et al., discloses the isolation of several isoforms of wild type Aequorea victoria GFP from varying cDNA libraries (see, Prasher et al., *Gene* 111:229-233 (1992), already of record). They report a comparative analysis of two of these clones, gfp2 and gfp10, which demonstrates that sequence differences in the GFP protein exist in the natural population. Notably, four conservative differences exist between gfp10, which is the sequence used in the instant application, and gfp2, isolated from a cDNA library generated from jellyfish collected at Friday Harbor in Washington. These conservative mutations consist of F100Y, T108S, L141M, and V219I (see Table I of Prasher et al.).

We further note, in this regard, that the Court of Appeals for the Federal Circuit has set forth that when accessible literature sources provide structural information for a biological macromolecule that literature also informs the judgment of a person of ordinary skill as to whether the applicants were in possession of the claimed invention at the time of filing. (See, *Falkner v. Inglis* (Fed. Cir. 2006) slip op at page 13, enclosed). Dopf and Horiagon additionally teach that residues 1 and 233-238 are not required for the characteristic emission and absorption spectra of native GFP (see, summary on page 39), evidencing that mutation of these residues, six of which are novel to this declaration, excluding K238, and five of which are further contained in the stretch of residues from 89 to 238, further excluding M1, would not effect the fluorescent activity of an AvGFP-rp (*see*, Dopf and Horiagon, *Gene* 173:39-44 (1996), enclosed

with IDS). In addition, Dopf et al. disclose at p. 363, first full paragraph of left column, under section entitled "Changes Without Spectral Effect," that Cys48 and Cys70 may be mutated to serine without spectral effect." Furthermore, Crameri et al. utilized molecular evolution to engineer improved GFP proteins with enhanced whole cell fluorescence signals. One such molecule, cycle 1, was determined to contain mutations in seven residues, of which two, S72 and K126, are additional to those described above and of which K126 is contained in the contiguous stretch of residues from 89 to 238 (*see*, Crameri et al., *Nat Biotechnol.* 14:315 (1996) at page 317, Figure 3, enclosed with IDS).

When the above art is considered together with the disclosed teachings of our application, which discloses how various classes of mutations affecting the various functional portions of the protein (e.g., the chromophore, the folding residues, the aggregation residues, external surface residues) can be combined, the disclosure readily supports the construction of an AvGFP-rp consisting of up to possibly as many as 54 mutations (77% sequence identity) (residues 1, 48, 70, 72, 100, 108, 126, 141, 219, 233-237, and the 38 listed above), with respect to the wild type sequence. Up to 40 of these residues (99, 100, 105, 108, 115, 123, 126, 141, 142, 144, 145,146, 147, 148, 149, 151, 153, 163, 167, 168, 170, 172, 175, 200, 202, 204, 205, 206, 208, 212, 219, 221, 223, 225, and 233-238) falling between the 150 contiguous residues stretching from 89 to 238 may be mutated to give as much as a 73% sequence identity.

Furthermore, one of ordinary skill in the art would easily be able to design a multitude of additional suitable conservative mutations, especially in residues either distal to the chromophore or whose side chains are solvent exposed and do not contribute directly to the electron environment of said chromophore. Sets of conservative amino acid substitutions are disclosed in U.S. Patent Application Serial No. 08/706,408 (now, U.S. Patent No. 5,625,048, already of record), which was incorporated by reference as discussed above. This expectation is in accord with the conservative mutations identified by Prasher et al. and Dopf et al., as discussed above. We also note that Heim et al., in their discussion of GFP mutants with altered spectral properties, disclose that they sequenced and recombined the sequences of these mutants to eliminate the neutral mutations which went unreported (*see*, Heim et al., *PNAS-USA* 91:12501

(1994) at p.12502 right column, starting at the ninth line of text, already of record). Practically as an aside, as discussed above, U.S. Patent Application Serial No. 08/337,915 sets forth seven mutations; K3R, D76G, F99I, N105S, E115V, T225S, and K238E which had *no* significant impact. The dearth of literature reporting on silent mutations can not be fairly construed to indicate that such do not exist or are not readily available. Rather, it is generally the case that generated conservative and or silent mutations are not likely to be reported for a number of reasons. Most importantly, such changes generated during mutational and screening analysis are not sought and rarely sequenced because the information often adds comparatively little to the understanding or the practical use of the fluorescent proteins.

With respect to the concern that some inoperative embodiments might lie in the above combinations, the specification indicates that mutations affecting the chromophore, protein folding, and protein binding generally are compatible with each other. It would further be expected that the *already*-identified silent and suggested conservative mutations, especially in the regions of the protein shown to be non-critical, would **not** be expected to very often eliminate a desired functionality.

Indeed, it appears that the Applicants' claims barely scratch the surface of the 'protein space' compatible with fluorescent protein activity. Matz et al. *Nature Biotechnology* 17: 969-973 (1999) identified six remote homologs of GFP from nonbioluminescent Anthozoa species. These fluorescent homologs had from 26 to 30% structural identity with GFP.

Accordingly, the Applicants submit that the specification and state of the art as of the time of filing would lead one of ordinary skill to recognize that the Applicants were well in possession of the claimed subject matter. Therefore, the Applicants respectfully request that the above grounds of rejection be reconsidered and withdrawn with regard to claims 79 to 81 and 94 to 96, and 98.

Response to the rejection of claims 85 to 90 and 99 under 35 U.S.C. §112, first paragraph, for an alleged lack of written description with respect to the 'linker' subject matter.

The Applicants respectfully traverse this grounds of rejection. As currently applied, the specification does comply with US patent law for description of a nucleic acid or

amino acid sequence. As threshold matter, the Applicants note that they have amended claims 79 to 8190 to set forth The base claims have also been amended to set forth that the donor moiety, the linker moiety, and the acceptor moiety are fused in a single amino acid sequence and to set forth that the linker *consists of* rather than *comprises* the recited number of amino acids. Accordingly, the linker subject matter is no longer 'open' as to the length of the linker.

The specification teaches that the principle function of the linker is to separate the two fluorescent domains of the construct. Applicants note that the functional and physical description of this criteria is also set forth in the theory and example in the specification at pages 20 and 21. Additionally, the theory underpinning the selection is described at length over pages 17 to 19.

More importantly, the specification discloses myriad linkers that do work. The specification in Table III at p. 24 sets forth some 12 linkers varying in length from 5 to over 30 amino acids in length. Additionally, the specification discloses some 8 linkers on page 21, (see Table II at p. 21) which vary from 12 to 25 amino acids in length. In addition, as noted in the previous response, that Exs. 1 and 2 exemplify the use of linkers over a broad size range from about 5 to about 25 amino acids in length (see p. 42). Accordingly, the Applicants believe the exemplification of the linker subject matter well exceeds that required to show possession of the invention pursuant to 35 U.S.C. §112 and accordingly respectfully request reconsideration and withdrawal of this grounds of rejection.

Response to the rejection of claims 79 to 81 and 94 to 96, and 98 for an alleged lack of enablement under 35 U.S.C. §112.

As set forth above, the base claims each set forth in part a tandem *Aequorea*-related fluorescent protein in which both fluorescent moieties contain, as defined in the detailed description of the invention, "...any contiguous sequence of 150 amino acids of the fluorescent protein (that) has at least 85% sequence identity with an amino acid sequence, either contiguous or non-contiguous, from the wild type *Aequorea* green fluorescent protein..." (see the paragraph bridging pages 14 and 15 of the application). An *Aequorea*-related fluorescent protein moiety (herein "*Av*GFP-rp") would therefore encompass any polypeptide having the recited

substitutions, and having at least 150 amino acids, with fewer than 23 amino acid differences, with respect to the wild type protein, in any contiguous 150 residues. In addition, we also contend, as discussed above with regard to written description, that one of ordinary skill in the art would easily be able to design a multitude of conservative mutations, especially in residues either distal to the chromophore or whose side chains are solvent exposed and do not contribute directly to the electron environment of said chromophore.

Standing by their earlier *Wands* analysis and with a minimal repetition, the Applicants next address subject matter pertaining to three of the *Wands* factors: the Teachings of the Specification, the State of the Art, and the Amount of Experimentation which would be routine in the field.

Teachings of the Specification

As discussed above, the specification teaches how to generate a tandem *Av*GFP-rp in which both fluorescent moieties contain 38 mutations (84% sequence identity), with respect to the wild type protein sequence, and which still retains useful, if not improved, fluorescent activity. This alone is enough for any person skilled in the art, which in this case would include protein design and evolution, to practice the full scope of the invention. Additionally, the specification teaches how to readily generate and select fluorescent proteins according to the claims. First, the incorporated Heim et al. 1994 reference teaches that mutants can be generated by standard means, namely hydroxylamine treatment or error prone PCR, and screened for different emission colors through the use of a xenon lamp and grafting monochromator (see, Heim et al., *PNAS* 91:12501-4 (1994), already of record. A second reference, which was incorporated at the end of the paragraph bridging pages 18 and 19, exemplified that hypothesis-driven site-specific mutation of a single residue can also generate a desired result (*see,* Heim et al., *Nature* 373:663-664 (1995),already of record). Heim et al. hypothesized that Ser 65, which becomes part of the p-hydroxybenzylideneimidazolinone chromophore, would undergo a dehydration event resulting in a vinyl side chain, which would contribute to the overall properties of said chromophore. Therefore, mutation of this residue, which would result in exclusion of the vinyl formation, should result in formation of a chromophore with altered

spectral properties. This is indeed what was found when Ser 65 was mutated and furthermore, the resulted mutant *Av*GFP-rp displayed advantageous spectral properties. It should be noted that this hypothesis was made prior to the determination of the high-resolution crystal structure of the protein, and therefore future site specific mutations should be considerably easier to design due to the availability of many such structures.

State of the Art and the Amount of Experimentation Considered Routine

The state of the art and the amount of experimentation are inter-related. The state of the pertinent experimental methodology is sufficiently advanced that what might otherwise be an enormous amount of experimentation is merely routine. In addition to the methods disclosed in the specification, Delagrave et al. teach methods for generating and screening such mutant *Av*GFP-rp's (see, Delagrave et al., *Biotechnology*, 13:151 155 (1995), already of record). This reference teaches use of optimized combinatorial mutagenesis techniques and Digital Imaging Spectroscopy to isolate mutant GFP proteins with advantageous properties (i.e., red-shifted excitation spectra). Using these methods, they were able to screen by fluorescence "Thousands of colonies on Petri dishes" (see the first complete paragraph on page 152) with "colony densities ranging from 100 to 900 colonies/plate" (see Digital Imaging Spectroscopy section on page 154). The result of the screen was the isolation of seven mutant proteins with the desired red-shifted excitation spectra, that each contained between three and four changes in the amino acid sequence.

Ehrig et al. teaches yet another method for generating mutant *Av*GFP-rp's with a desired property (see, J: Ehrig et al., *FEBS Letters* 367:163-166 (1995), already of record). In this paper, Ehrig et al. was able to generate mutant proteins that differentially excited at one, but not the other, of the two characteristic maximal excitation wavelengths of wild type GFP. In order to do so, they randomly mutagenized GFP cDNA through use of the *E. coli* XL1-Red strain and subsequently screened approximately 200,000 colonies for the desired separation of excitation wavelengths.

Thus, the specification and prior art provide at least five different methods for generating additional point mutants of *Av*GFP-rp's. These references demonstrate that any

person skilled in the art to which it pertains, or with which it is most nearly connected, should easily be able to generate additional mutations to the mutant *Av*GFP-rp moieties disclosed in the specification. Furthermore, these were able to generate their respective mutations without the aid of a high-resolution crystal structure as incorporated into the specification by reference.

Additionally, as discussed above with reference to the written description rejection, the specification and the state of the art provide a great many more silent mutations which are suitable for use in making the constructs according to the claims.

Indeed, the subsequent work of Campbell et al., who engineered *33* mutations into a GFP-related protein isolated from *Discosoma* coral, dsRed shows that the amount of experimentation required to practice the claimed invention is well within the capacity of those in the broader field (Campbell et al., *PNAS* 99:7977-82, 2002, enclosed with IDS, see Abstract,). The Campbell et al. mutant had about 85% sequence homology to the wild type dsRed protein and retained useful fluorescent activity, including advantageous shifts in its emission and excitation peaks, with respect to the wild type protein.

In view of the extensive disclosure of compatible mutations in the specification, the crystallographic and functional analyses provided therein, the advanced state of the art with respect to the manipulations needed to practice the claimed invention, and the relative simplicity with which mutants may be screened in truly enormous numbers, and the ability of others in the field to obtain useful fluorescent proteins with 85% substitutions, the Applicants submit that the claimed invention can be practiced with an amount of experimentation which would be routine in the art.

Accordingly, the Applicants respectfully request that the above grounds for rejection be reconsidered and withdrawn.

Response to the rejection of claims 88 to 90 and 99 under 35 U.S.C. §112, first paragraph, for an alleged lack of enablement with respect to the 'linker' subject matter.

The Applicants respectfully traverse this grounds of rejection. To the extent that this ground of rejection is based upon the 'linker' subject matter, this rejection was based upon the 'open' nature of the transition term "comprising" and a misinterpretation of the specification.

The Applicants address each concern in turn. As currently applied, the specification does comply with US patent law for enablement of an amino acid sequence. To address the first concern, in the spirit of expediting prosecution, the Applicants have amended claims 79 to 81 to set forth the 'closed' transition term *consists of* in place of the 'open' transition term *comprises* with respect to the recited number of amino acids. Applicants note that the recital of *is* in claim 99 is also 'closed'.

Turning to the second concern, the Action appears to misconstrue the passage defining 'moiety' at page 8:

> Moiety" refers to the radical of a molecule that is attached to another moiety. Thus, a "fluorescent protein moiety" is the radical of a fluorescent protein coupled to the linker moiety. By the same token, the term "linker moiety" refers to the radical of a molecular linker that is coupled to both the donor and acceptor protein moieties.

The Action construes that the above passage appears to define a linker moiety as "encompassing those molecules that can be fluorescent in the same manner as the donor and acceptor moieties.' The proffered interpretation is simply wrong and is not fairly supported by the cited passage, especially when read in light of the remainder of the specification or the preceding sentences. The passage is more reasonably understood to indicate that the 'linker moiety" is a linker radical that serves to join the acceptor and donor moieties which are described in the immediately preceding sentence as each being coupled to the linker moiety. These two sentences need to be read consistently.

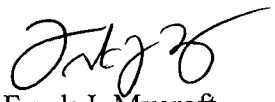
Accordingly, in light of the above clarification and the limitation of the base claims to the linker lengths deemed to be enabled by the Examiner, the Applicants respectfully request that the above grounds for rejection be reconsidered and withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



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